Table 3. *In vitro* antibacterial activity of *N*-alkyl vancomycins.

| Compound | MIC (μg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S.A. 1 | S.A. 2 | S.A. 3 | S.A. 4 | S.E. 1 | S.E. 2 | S.Py. | S.Pn. | S.D. 1 | S.D. 2 |
| **47** R=$C_3H_5$, $R_1$=H | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 0.5 | 2 | 4 |
| **48** R=$R_1$=$C_3H_5$ | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 4 |
| **49** R=H, $R_1$=*n*-$C_5H_{11}$ | 0.5 | 1 | 1 | 1 | 2 | 1 | 0.5 | 0.125 | 1 | 2 |
| **50** R=*n*-$C_6H_{11}$, $R_1$=H | 2 | 2 | 2 | 2 | 4 | 2 | 1 | 0.5 | 2 | 8 |
| **51** R=H, $R_1$=*n*-$C_6H_{13}$ | 0.5 | 1 | 1 | 0.5 | 2 | 1 | 0.5 | 0.125 | 1 | 1 |
| **52** R=*n*-$C_6H_{13}$, $R_1$=H | 1 | 1 | 2 | 2 | 4 | 2 | 1 | 0.5 | 2 | 4 |
| **53** R=H, $R_1$=*n*-$C_9H_{19}$ | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.125 | 0.25 | 0.25 | 0.5 |
| **54** R=$R_1$=*n*-$C_9H_{19}$ | 1 | 1 | 1 | 2 | 4 | 2 | 1 | 2 | 1 | 2 |
| **55** R=H, $R_1$=$(C_6H_5)_2CHCH_2$ | 0.5 | 0.5 | 1 | 1 | 4 | 1 | 0.25 | 0.25 | 1 | 4 |
| **56** R=$(C_6H_5)_2CHCH_2$, $R_1$=H | 1 | 2 | 2 | 2 | 4 | 2 | 1 | 1 | 2 | 8 |
| **57** R=H, $R_1$=$CH_3S(CH_2)_3$ | 0.5 | 1 | 1 | 0.5 | 2 | 1 | 0.5 | 0.25 | 1 | 4 |
| **58** R=$CH_3S(CH_2)_3$, $R_1$=H | 2 | 8 | 8 | 2 | 8 | 8 | 2 | 2 | 8 | 32 |
| **59** R=H, $R_1$=$(C_6H_5)_2CHCH_2$ | 0.25 | 0.25 | 0.5 | 0.5 | 2 | 0.5 | 0.25 | 0.06 | 0.25 | 1 |
| **60** R=$(C_6H_5)_2CHCH_2$, $R_1$=H | 4 | 4 | 4 | 4 | 8 | 8 | 4 | 2 | 4 | 8 |
| **61** R=H, $R_1$=*p*-$CH_3SC_6H_4CH_2$ | 0.25 | 0.25 | 0.5 | 0.25 | 1 | 0.5 | 0.25 | 0.125 | 0.25 | 1 |
| **62** R=*p*-$CH_3SC_6H_4CH_2$, $R_1$=H | 2 | 2 | 2 | 2 | 8 | 2 | 2 | 2 | 2 | 8 |
| **63** R=H, $R_1$=*p*-$C_4H_9C_6H_4CH_2$ | 0.125 | 0.06 | 0.06 | 0.06 | 0.125 | 0.125 | 0.125 | 0.125 | 0.06 | 0.25 |
| **64** R=$R_1$=*p*-$C_4H_9C_6H_4CH_2$ | 4 | 4 | 4 | 4 | 8 | 4 | 2 | 2 | 2 | 4 |
| **65** R=H, $R_1$=*p*-$C_4H_9OC_6H_4CH_2$ | 0.125 | 0.125 | 0.125 | 0.125 | 0.25 | 0.125 | 0.06 | 0.06 | 0.06 | 0.25 |
| **66** R=$R_1$=*p*-$C_4H_9OC_6H_4CH_2$ | 4 | 4 | 4 | 4 | 8 | 4 | 2 | 8 | 4 | 8 |

Abbreviations: See Table 1.



Table 4. *In vivo* antibacterial activity and pharmacokinetics of *mono-N*-alkyl vancomycins.

| Compound (R=H, $R_1$=as shown) | MIC (μg/ml) | | | $ED_{50}$ (mg/kg×2, sc) | | | Serum (iv, rat) | |
|---|---|---|---|---|---|---|---|---|
| | S.A.1 | S.Py. | S.Pn. | S.A.1 | S.Py. | S.Pn. | $T_{1/2}$ (hours) | 5 minutes conc (mg/ml) |
| **11** *n*-$C_4H_9$ | 1 | 1 | 0.5 | 6.3 | 1.9 | 1.3 | 0.5 | 45 |
| **51** *n*-$C_6H_{13}$ | 0.5 | 0.5 | 0.125 | 3.6 | 1.1 | 0.9 | 0.68 | 109 |
| **17** *n*-$C_8H_{17}$ | 0.25 | 0.125 | 0.125 | 3.4 | 0.9 | 0.4 | 2.1 | 256 |
| **5** *n*-$C_{10}H_{21}$ | 0.13 | 0.06 | 0.13 | 1.8 | 0.65 | 0.68 | 3.4 | 203 |
| **67** *n*-$C_{12}H_{25}$ | 0.25 | 0.125 | 0.25 | 5.7 | 0.3 | 0.4 | 2.9 | 254 |
| **55** $(C_2H_5)_2CHCH_2$ | 0.5 | 0.25 | 0.25 | 1.8 | 1.1 | 1.7 | 1.7 | 95 |
| **29** $C_6H_{11}CH_2$ | 0.5 | 0.5 | 0.25 | 1.8 | 1.4 | 0.9 | — | — |
| **20** $(CH_2)_5OH$ | 1 | 1 | 1 | 5.2 | 5.8 | 1.8 | — | — |
| **23** $C_2H_5O(CH_2)_3$ | 1 | 0.5 | 0.5 | 6.2 | 4.3 | 2.0 | — | — |
| **57** $CH_3S(CH_2)_3$ | 0.5 | 0.5 | 0.25 | 3.7 | 1.4 | 1.5 | 0.68 | 97 |
| **32** $C_6H_5CH_2$ | 0.06 | 0.06 | 0.015 | 0.8 | 1.0 | 0.9 | 1.84 | 89 |
| **59** $(C_6H_5)_2CHCH_2$ | 0.25 | 0.25 | 0.06 | 0.6 | 1.3 | 0.8 | 1.49 | 116 |
| **35** $C_6H_5(CH_2)_2$ | 0.5 | 0.5 | 0.25 | 1.0 | 3.1 | 1.0 | — | — |
| **38** $C_6H_5(CH_2)_3$ | 0.5 | 0.25 | 0.25 | 1.1 | 1.6 | 1.2 | 1.74 | 79 |
| **68** $C_6H_5CH(CH_3)CH_2$ | 0.5 | 0.03 | 0.125 | 1.8 | 0.8 | 0.9 | — | — |
| **69** *p*-$(CH_3)_2CHC_6H_4CH_2$ | 0.25 | 0.25 | 0.25 | 1.1 | 0.9 | 0.9 | 1.6 | 86 |
| **63** *p*-$C_4H_9C_6H_4CH_2$ | 0.125 | 0.125 | 0.125 | 0.7 | 0.4 | 0.8 | 5.4 | 156 |
| **70** *p*-$C_2H_5C_6H_4CH_2$ | 0.125 | 0.125 | 0.125 | 0.6 | 0.8 | 0.6 | — | — |
| **71** *p*-$C_6H_{13}C_6H_4CH_2$ | 0.5 | 0.25 | 0.25 | 0.19 | 0.62 | 0.23 | — | — |
| **44** *p*-$CH_3OC_6H_4CH_2$ | 0.5 | 0.25 | 0.25 | 1.1 | 0.6 | 2.3 | — | — |
| **65** *p*-$C_4H_9OC_6H_4CH_2$ | 0.125 | 0.06 | 0.06 | 0.7 | 0.5 | 0.6 | 2.4 | 205 |
| **72** *p*-$C_6H_{13}OC_6H_4CH_2$ | 0.125 | 0.06 | 0.06 | 0.6 | 0.9 | 0.7 | — | — |
| **73** *p*-$C_6H_{13}OC_6H_4CH_2$ | 0.25 | 0.25 | 0.25 | 0.2 | 0.2 | 0.2 | — | — |
| **61** *p*-$CH_3SC_6H_4CH_2$ | 0.25 | 0.25 | 0.125 | 1.1 | 2.1 | 0.9 | — | — |
| **74** *p*-$CNC_6H_4CH_2$ | 0.5 | 0.25 | 0.125 | 1.8 | 2.5 | 1.2 | 0.68 | 112 |
| **41** *p*-$HOC_6H_4CH_2$ | 0.5 | 0.5 | 0.06 | 0.9 | 1.1 | 1.2 | 0.67 | 145 |
| **75** *p*-$(CH_3)_2NC_6H_4CH_2$ | 0.5 | 0.25 | 0.5 | 1.3 | 3.1 | 1.9 | 2.0 | 119 |
| **76** *p*-$(C_2H_5)NC_6H_4CH_2$ | 0.25 | 0.25 | 0.125 | 1.1 | 0.7 | 1.9 | 5.8 | 110 |
| **77** *p*-$CH_3CONHC_6H_4CH_2$ | 0.5 | 0.5 | 0.25 | 1.7 | 4.9 | 3.7 | — | — |
| **78** *p*-$BrC_6H_4CH_2$ | 0.25 | 0.25 | 0.03 | 1.3 | 0.8 | 0.4 | 1.7 | 136 |
| **79** *m*-$BrC_6H_4CH_2$ | 0.5 | 0.25 | 0.125 | 0.9 | 1.2 | 0.7 | 1.6 | 120 |
| **80** *o*-$BrC_6H_4CH_2$ | 1 | 0.5 | 0.25 | 1.4 | 1.0 | 1.3 | — | — |
| **81** *p*-$ClC_6H_4CH_2$ | 0.5 | 0.25 | 0.03 | 0.8 | 1.8 | 0.7 | 2.0 | 188 |
| **82** 2,6-di$ClC_6H_3CH_2$ | 0.5 | 0.5 | 0.5 | 2.5 | 1.8 | 3.1 | — | — |
| **83**  | 1 | 1 | 0.5 | 1.3 | 1.9 | 3.3 | — | — |
| **84**  | 1 | 0.5 | 0.25 | 1.4 | 1.8 | 2.5 | — | — |
| **85**  | 1 | 0.5 | 0.25 | 1.9 | 6.8 | 3.1 | 0.43 | 117 |

Abbreviations: See Table 1.

representative structural types were also undertaken. In the aliphatic straight chain series, increasing the chain length enhances activity and the optimum chain length seems to be the $C_{10}$ analog. Branching the aliphatic side chains, or substituting with oxygen or sulfur (compounds **20**, **23**, **29**, **55** and **57**) does not seem to increase activity. The benzyl derivative **32**, and the benzyl derivatives substituted at the 4-position with an aliphatic side chain, especially compounds **63**, **65**, **70** and **76**, are more active

Table 5. MIC[a] and MBC[a] for *Streptococcus* sp. (Group D).

| Compound | *Streptococcus* sp. (Group D) strain No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 238 | | Guze | | Mitis | | Shrigley | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Vancomycin | 2 | >128 | 0.25 | >128 | 0.25 | >128 | 1.0 | >128 |
| 5 | 0.03 | 64 | 0.008 | 64 | 0.008 | >128 | 0.008 | >128 |

[a] μg/ml.

Table 6. Rat endocarditis using *Streptococcus faecium* X66.

| Compound | Dosage (mg/kg×28) | 5th Day[a] | | 9th Day[a] | | 16th Day[a] | |
|---|---|---|---|---|---|---|---|
| | | % Cured | % With $10^4$ drop | % Cured | % With $10^4$ drop | % Cured | % With $10^4$ drop |
| Vancomycin | 20 | 33 | 33 | 100 | 100 | 100 | 100 |
| 32 | 20 | 100 | 100 | 66 | 100 | 100 | 100 |

[a] Days after initiation of treatment.

than the aliphatic series in the *in vivo* models. The most active compounds are the octylbenzyl (71) and octyloxybenzyl (73), derivatives. Substitution at the *para*-position of the benzyl moiety with hetero atoms like oxygen, sulfur, nitrogen or halogens does not seem to enhance activity. The heterocyclic analogs 83, 84 and 85 also have no advantage over the benzyl analog 32. In general, these mono-*N*-alkyl vancomycins have more favorable pharmacokinetics than the parent vancomycin. Butyl and butyloxy derivatives, 63 and 65, have elimination half-lives of 5.4 and 2.4 hours in rats, respectively.

Table 7. Rat descending pyelonephritis with *Streptococcus faecalis* Guze.

| Compound | Dosage (mg/kg×12) | % Cured | % With $10^4$ log drop |
|---|---|---|---|
| Vancomycin | 10 | 80 | 80 |
| 63 | 10 | 100 | 100 |
| 65 | 10 | 90 | 90 |
| 32 | 10 | 60 | 70 |
| Vancomycin | 2 | 10 | 50 |
| 63 | 2 | 70 | 100 |
| 65 | 2 | 40 | 80 |
| 32 | 2 | 10 | 70 |

Since several of the *N*-alkyl vancomycins were more active than the parent antibiotic, further evaluations of representative members of the *N*-alkyl vancomycin series were undertaken. Comparison between the MIC and MBC of the mono-*N*-decyl vancomycin 5 and vancomycin for four strains of Group D *Streptococcus* sp. showed that both compounds were bacteriostatic. The mono-*N*-benzyl vancomycin 32 and the parent vancomycin were examined in the rat endocarditis model using *Streptococcus faecium* X66. Both compounds had comparable activity in this test.

Finally, the mono-*N*-benzyl (32), mono-*N*-butylbenzyl (63), and mono-*N*-butyloxybenzyl (65) derivatives were evaluated in the descending pyelonephritis rat model with *Streptococcus faecalis* Guze. The order of activity in the above test of the *N*-alkyl vancomycins were: Mono-*N*-butylbenzyl (63) > mono-*N*-butyloxybenzyl (65) > mono-*N*-benzyl (32) = vancomycin.

Several compounds in the *N*-alkyl vancomycin series were more active than vancomycin. Octylbenzyl (71), octyloxybenzyl (73), butylbenzyl (63), butyloxybenzyl (65) and benzyl (32) derivatives had the best activity and are up to five times more active than vancomycin.



## Experimental

### General Procedure for the Preparation of *N*-Alkyl Vancomycins

The desired aldehyde was added to a solution of vancomycin base in DMF. The solution was stirred and a slight excess of sodium cyanoborohydride was added to the intermediate SCHIFF's base formed. After completion of the reaction, the mixture of products was separated by chromatography. A shorter reaction time and an equimolar amount of the aldehyde favored the formation of mono-*N*-alkyl vancomycins. Longer reaction time and an excess of aldehyde gave the di-*N*-alkyl derivative as the major product.

Example: Vancomycin base (5 g, 3.5 mmol) was dissolved in DMF and *n*-decyl aldehyde (0.7 ml, 3.72 mmol) was added. The reaction mixture was stirred for 2 hours in a 70°C oil bath.

Sodium cyanoborohydride (275 mg, 4.4 mmol) was added to the solution containing the SCHIFF's base formed *in situ*. The reaction mixture was stirred for 2 hours in the 70°C oil bath, then cooled to room temperature and the DMF evaporated to dryness. The reaction mixture was purified by reverse-phase HPLC using a Waters Prep Pak/500 column and an acetonitrile - water gradient. The eluates were monitored by analytical HPLC using UV detection at 280 nm. Appropriate fractions were pooled and 794 mg of mono-*N*-decyl vancomycin 5 was obtained. The identity of the product was confirmed by FAB-MS.

Chromatography: The conditions used for the analytical and preparative HPLC were described earlier.[8]

FAB-MS: FAB-MS spectra were determined using a VGZ AB-3F mass spectrometer. Samples were dispersed in thioglycerol and introduced into the spectrometer on a cooled FAB target.

### Antibacterial Activity *In Vitro*

The MICs for the aerobic bacteria strains were determined in an agar dilution assay. Mueller-Hinton agar containing 1% supplement C (Difco Laboratories, Detroit, Michigan) was used. The dilutions of the antibiotics were made in water and mixed with the melted agar prior to pouring the plates. The various bacteria were inoculated onto the medicated plates using a Cotlara replicator at an inoculum of $10^4$ cfu/spot. The plates were then incubated for 20~24 hours at 35°C. End points were read to discrete colonies.

### Antibacterial Activity *In Vivo*

The therapeutic efficacy of the vancomycin derivatives were determined in standard mouse protection tests. An experimental systemic infection was produced using ICR random sex mice (Harland Laboratories, Cumberland, Indiana), by intraperitoneal inoculation of a suitable diluted broth culture of the infecting organism. The test compounds (*N*-alkyl vancomycins) were administered subcutaneously at 1 and 5 hours post-infection. Five 2-fold dilutions of each antibacterial agent was tested and there were eight mice for each dose level. All the mice were observed for a period of 7 days, after which the effective dose ($ED_{50}$) was calculated by the method of REED and MUENCH.[9] Under the conditions of the test, all infected and untreated mice died within 48 hours.

### MIC and MBC Determination

Determination of the broth MIC was carried out in Mueller-Hinton broth supplemented with 50 mg $Ca^{2+}$ and 25 mg $Mg^{2+}$ per liter in autotiter plates. To determine the MBC (99.99 killing) of the antibiotic, subcultures onto Mueller-Hinton agar without antibiotics were made using a 10-μl loopful from those dilutions on the MIC plates which failed to show macroscopic turbidity. These plates were read after 72 hours of incubation.

### Production and Treatment of Endocarditis

Groups of 8~10 female Sprague Dawley rats weighing 200~220 g were anesthetized and the right carotid artery was exposed. A silastic catheter was inserted *via* the right carotid artery through the aortic valve into the left ventricle of the heart and secured in place with a silk ligature. Forty eight hours after catheterization, the rats were injected *via* the tail vein with 0.5 ml of *S. faecium* X66 culture containing $5\times10^8$ organisms per ml. Subcutaneous therapy with the test antibiotics began